THE EVOLUTION OF SARCOCYSTIS MURIS IN THE INTESTINAL CELLS OF THE MOUSE.

(Preliminary Note.)

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As long ago as 1903, Minchin (1903, p. 308), speaking of the Sarcosporidia, observes that "there is still much to be made out about these interesting parasites, and the field is one ripe for investigation."

Since that time it cannot be said that our knowledge of the group has been materially increased. A number of papers on the Sarcosporidia have indeed been published, but these have been concerned with the character of the spores and cysts rather than with any attempts to elucidate the life history of this group of the Protozoa. The exception is a contribution by Erdmann (1910, p. 377), the results of which are summed up and commented upon by Minchin (1912, pp. 421, 422) as follows:

"According to Erdmann, the spore germinates in the intestine of the new host, and the first act in the process is the liberation from the spore of its toxin, sarcocystine, which causes the adjacent epithelium of the intestine to be thrown off. At the same time an amœbula is set free from the spore; and, owing to the intestine being denuded of its lining epithelium, the amœbula is able to penetrate into the lymph-spaces of the submucous coat and establish itself there. Before this happens, however, the metachromatinic grains of the spore disappear, and it is suggested that this disappearance is related to the secretion of the sarcocystine, and that the toxin is contained in the metachromatinic grains. If, however, a polar capsule be discharged during the germination of the spore, as in other Cnidosporidia, it might well be that the toxin is contained in the polar capsule and is set free by its discharge, like the poison in the nematocysts of the Cœlentera. However that may be, it would appear as if the sarcocystine were a weapon, as it were, the function of which is to facilitate the invasion of the germ, the amœbula, by destroying the lining epithelium of the gut.

The liberation of the amœbula from the spore initiates the first period of the development, which is passed in the lymph-spaces of the intestine, and which lasts, according to Erdmann, some twenty-eight to thirty days. Analogy with other Neosporidia would lead us to identify this with the planont-phase, initiated, possibly, by sexual processes between different amœbulæ and subsequent active multiplication. The second period of the development begins with

the penetration of the amœbula into a muscle-fiber, in which the parasite grows into a Miescher's tube and forms spores."

The present writer has for some time been in possession of material which illustrates the earlier stages of the cycle of *Sarcocystis muris* in the mouse, but sufficient time has not been available completely to work out this cycle in all of its details. Since, however, Erdmann's conclusions are largely erroneous, and since, moreover, they are becoming incorporated into general works on the Protozoa, it has been considered desirable to publish a brief notice giving the essential facts discovered, which are of considerable theoretical interest. A short note bearing on this matter was published in *Science* (1913, n. s., v. 37, p. 498) last year, but this did not touch upon the more important of the discoveries made.

As stated in the note which appeared in *Science*, the spore when in the lumen of the intestine of the mouse does not set free an amœbula, since it is itself a naked mass of protoplasm. What actually takes place is that the spore, when in the intestine of the mouse, becomes endowed with the ability to display very energetic twisting and boring movements, by virtue of which it forces its way into a cylinder cell of the intestinal epithelium, and there comes to rest. This takes place within $2\frac{1}{2}$ hours after the infecting feed, and possibly much earlier.

The typical spore of *Sarcocystis muris*, which has been figured a number of times in the literature, is a banana-shaped organism about 12μ long. Spores of this sort are found both free in the lumen and in the cylinder cells in mice killed and examined at appropriate periods after the inoculative feed. Besides these, however, others occur, such as are shown in Plate XV, figs. 1 and 2. These are oval bodies, generally about half as long as the typical spore. The cytoplasm has a considerable affinity for chromatin stains and consists of a dense spongioplasm. The nucleus is vesicular and more conspicuous than it is in the typical spores. It apparently always contains either a feebly developed nuclear net (fig. 3) or a karyosome or both, but these last-named structures require heavy staining for their demonstration, and in moderately or lightly stained material the appearance is as shown in figs. 1 and 2.

Figures 2 and 3 represent conditions found in a mouse killed about $2\frac{1}{4}$ hours after feeding. Since, however, the spores in the lumen of the intestine of this mouse are in precisely the same state as those illustrated in fig. 2, the presumption is that these latter have only been in the cells a very short time. Moreover, the intracellular

parasites both in $2\frac{1}{4}$- and $3\frac{1}{4}$-hour stages have, at least in a certain proportion of cases, undergone conspicuous changes. These changes consist in a gradual diminution of the quantity of cytoplasm, which seems either largely or completely to disappear, while concommitantly there is an increase in size and complexity of the nucleus. There is in this way produced a parasite such as is shown in fig. 4, which, so far as both its history and appearance go, is only the nucleus of the original spore. The stage here represented is especially characteristic of the period about six hours after feeding. It may, however, be stated that it is not certain that all of the parasites which invade the cells suffer this loss of the cytoplasm.

In mice killed nine hours after feeding, this same stage (as shown in fig. 4) may also be found, but it is no longer abundant. This period in the evolution of the parasite, that is, nine hours after feeding, is characterized by a great variety of conditions, of which the majority are difficult to interpret. But by this time it has become evident that the parasites are separating into two categories, which become more and more sharply differentiated as time passes, and which reach their full culmination at the end of 18 hours. The end products of these two lines of evolution are shown in figs. 9 and 11, and the interpretation placed upon them is that they are respectively males and females.

The male elements appear to arise from forms like that shown in fig. 4. These, which apparently consist of only the nucleus of the original spore, show a karyosome, and a nuclear net which here and there supports little aggregates of chromatin. Later stages (fig. 5) show a greater quantity of chromatin, but the karyosome has disappeared. Figure 5 is to be taken merely as representing one of a number of forms which, while differing greatly in detail, agree in that each possesses a nuclear net which supports a quantity of chromatin. In some cases the chromatin occurs in a much coarser form than that shown in fig. 5, whereas in others it is present in very minute granules distributed throughout the entire extent of a finely meshed net.

Eventually, however, a stage is reached such as is shown in fig. 7. This consists of an oval body with a stringy matrix and a row of granular aggregates arranged around the periphery. These granular aggregates become more and more compact until finally they come to consist of solid, round balls of deeply staining chromatin (fig. 8). These balls, in their turn, elongate and transform themselves into bodies such as are shown in fig. 9, which can scarcely be other

than microgametes. As seen in sectioned material, the microgametes are from 2 to 2.5 microns long, with both ends pointed, but one noticeably broader than the other. They are characterized by an intense affinity for chromatin stains. Stages such as these may occur as early as nine hours, but it is not until later that they become abundant. They reach their full development at the end of 18 hours, and, so far as my studies have yet gone, are no longer present at the end of 24 hours.

It is only in their later developmental phases that the females can be picked out with any certainty. They are illustrated in figs. 10 and 11, which show oval elements containing a vesicle in which is a chromatin body. In the 18-hour stage all of the parasites present, with a certain exception to be noted below, are either in the condition shown in figs. 7, 8, and 9, or that shown in figs. 10 and 11. As was stated above, however, the parasites taken to be early male stages were apparently only nuclei, since if any cytoplasm were present it was reduced to an extremely fine peripheral film. This conclusion was based not only upon the history of these bodies, but also upon their appearance. On the other hand, the bodies shown in figs. 10 and 11 have all the appearance of complete cells, with a considerable bulk of cytoplasm. It may then be that from the very outset some of the parasites retain a part or the whole of their cytoplasm, these being destined to produce the macrogametes. This surmise receives a certain amount of support from what is seen in fig. 6. This parasite appears to have retained at least the greater part of its cytoplasm. But we have here the representative of a condition found nine hours after feeding, whereas the loss of cytoplasm on the part of those parasites which suffer this deprivation may be completed as early as $2\frac{1}{2}$ to 3 hours. It may then be suggested that fig. 6 represents an early female stage, and if this be so it would follow that the females retain most if not all of their cytoplasm. It may also be noted that in the periods from 9 to 18 hours parasites which are clearly females show phenomena which suggest maturation.

Finally, in the 18-hour period there is to be found the condition illustrated in fig. 12. This shows a parasite in all respects like figs. 10 and 11 except for the presence in the cytoplasm of a sharply staining chromatin body. It does not seem unreasonable to look upon this as a microgamete which has fertilized the macrogamete.

Bibliography.

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Explanation of Plate XV.

The figures were in all cases made by the author from camera outlines, and later copied in ink by Mr. Haines, artist of the Bureau of Animal Industry. The optical system consisted of a 2-mm. apochromatic objective and No. 18 compensating eyepiece, yielding a magnification of about 3,530 diameters. In reproduction the drawings have been reduced in the ratio of 3 to 2, and hence are about 2,350 times larger than the actual object.

Fig. 1.—Shortened spore free in the lumen of the intestine. Two to two and one-half hour period. Giemsa stain.

Fig. 2.—Spores in the cylinder cells of the host. Two to two and one-half hour period. Giemsa stain.

Fig. 3.—Spore in a cylinder cell of the host. Two to two and one-half hour period. Wright's stain.

Fig. 4.—Form from which the males are supposed to arise. Taken from a nine-hour period. Delafield's hæmatoxylin and eosin.

Fig. 5.—Supposed early male stage. Nine-hour period. Iron hæmatoxylin and acid fuchsin.

Fig. 6.—Supposed early female stage. Nine-hour period. Iron hæmatoxylin and acid fuchsin.

Fig. 7.—Microgametocyte with granular nuclei. Eighteen-hour period. Wright's stain.

Fig. 8.—Microgametocyte with solid nuclei. Taken from a mouse killed nine hours after feeding, in which this stage is very rare. Iron hæmatoxylin and acid fuchsin.

Fig. 9.—Microgametocyte in which the microgametes are fully ripe. Eighteen-hour period. Wright's stain.

Fig. 10.—Macrogamete. Seventeen-hour period. Iron hæmatoxylin and acid fuchsin. Stages such as this are more commonly found in the subepithelial spaces than in the cells themselves.

Fig. 11.—Macrogamete. Eighteen-hour period. Wright's stain.

Fig. 12.—Supposed fertilization. Eighteen-hour period. Wright's stain. Parasites showing the supposed fertilization were not found in the cells themselves, but in the spaces beneath the epithelium.